Commentary

Global regulation of gene expression

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Microorganisms such as *Escherichia coli* live in environments subject to rapid changes in the availability of the carbon and nitrogen compounds necessary to provide energy and building blocks for the synthesis of cell material. Their survival depends on their ability to regulate the expression of genes coding for the enzymes and transport proteins required for growth in the altered environment. The availability of microarrays of the entire genome of *E. coli* has made it possible to measure the expression of all genes by determining the levels of the corresponding species of RNA. As reported in this issue of PNAS, Sydney Kustu, Robert A. Bender, and their coworkers (1) have used this method to identify all of the genes whose expression is activated in response to the replacement of the preferred nitrogen source, ammonia, by a non-preferred source of nitrogen (nitrogen regulation) (2). Their study revealed that the products of many genes that had previously not been known to be subject to nitrogen regulation are responsible for the scavenging of amino acids and peptides, among them the dipeptide D-alanyl-D-alanine. Under conditions of stress, probably including nitrogen limitation, *E. coli* switches the mechanism for crosslinking the peptidoglycan layer of the cell wall to one that releases D-alanyl-D-alanine into the growth medium. The activation of the expression of the nitrogen regulated *ddp* operon, comprising the genes for the D-alanyl-D-alanine dipeptidase transport system and for the D-alanyl-D-alanine dipeptase, enables the cells to retrieve the excreted dipeptide and to use it for the replenishment of its nitrogen store. More importantly, the design of their study should serve as a model for future attempts to use microarrays to investigate the global regulation of gene expression.

E. coli, like many other microorganisms, grows well in a medium containing glucose as sole source of energy and carbon and an ammonium salt as sole source of nitrogen. It can also grow, although more slowly, when one of a number of amino acids is substituted for ammonia as source of nitrogen. The close relative of *E. coli*, *Klebsiella aerogenes*, can in addition use nitrate or dinitrogen as sole source of energy (2). The utilization of these alternative sources of nitrogen requires a number of enzymes and permeases, which play no role when ammonia is available. The efficient incorporation of ammonia into glutamate and glutamine, the precursor molecules for all cellular nitrogen compounds, is responsible for its role as the preferred source of nitrogen. The decline in the intracellular concentration of glutamine, resulting from the replacement of ammonia by another source of nitrogen, is the signal for the activation of the expression genes subject to nitrogen regulation (3). In response to this signal, the kinase NtrB, the product of the *glnL* gene, phosphorylates the response regulator NtrC, the product of the *glnG* gene, and thus enables it to activate transcription at the σ^{54}-dependent promoter of the *glnALG* operon (4). The increase in the level of the *glnA* product, the enzyme glutamine synthetase compensates in part for the reduced availability of ammonia and the increase in the level of NtrC-phosphate results in increased expression of nitrogen-regulated genes and operons with σ^{54}-dependent promoters whose products have the potential to increase the intracellular concentration of glutamine (2). One of these genes is *nac,* whose product in turn activates transcription of a set of nitrogen-regulated genes at $\sigma^{70}-$dependent promoters (5).

An essential feature of this regulatory mechanism is that an increase in the intracellular concentration of glutamine results in the dephosphorylation of NtrC-phosphate by NtrB, which in this condition acts as a phosphatase (4). Consequently, the rate of glutamine production from the non-preferred nitrogen source must be slower than the rate of its production from ammonia to maintain the low intracellular concentration of glutamine required for the activation of the expression of nitrogen-regulated genes. Thus, nitrogen regulation can only ameliorate, but not cure, ammonia deficiency. Replacement of ammonia by another source of nitrogen will therefore result in effects that are not directly related to nitrogen regulation, but rather result from the slower growth rate. For example, the decline in the rate of synthesis of proteins and nucleic acids because of the reduction in the intracellular concentration of glutamine will lead to excessive accumulation of the products of glucose catabolism, which in turn is likely to affect the expression of genes whose product are involved in this pathway. Therefore, using microarrays to compare the expression of genes in cells using ammonia with those of cells using another source of nitrogen would not unequivocally identify the genes whose expression is subject to nitrogen regulation.

Monod recognized the significance of this problem for the study of the regulation of gene expression in intact cells in 1952 (6). He pointed out that such a study required a condition of "gratuity," that is, a condition where ideally the expression of the regulated genes does not affect the physiology of the cell. The authors of the current study (1) achieved a condition of gratuity by comparing the expression of nitrogen-regulated genes in cell populations growing in the same medium with ammonia as sole source of nitrogen. This was possible because three mutants with alterations in the genes responsible for nitrogen regulation were available. One mutant lacked the *glnG* gene and therefore was unable to produce the NtrC required for the activation of the transcription of nitrogen-regulated genes; the second mutant had an alteration in the *glnL* gene, *glnL(Up)*, resulting in an altered NtrB product that phosphorylated NtrC even when ammonia served as source of nitrogen; the third mutant carried the *glnL(Up)* gene, as well as an insertion in the *nac* gene, preventing its expression (2). A comparison of the RNA levels in these three types of cells growing with ammonia as source of nitrogen made it possible to identify all of the genes activated by NtrC and thus subject to nitrogen regulation and to distinguish those that were directly activated by NtrC from those that were indirectly activated by NtrC as a consequence of its activation of the expression of the *nac* gene.

Future investigators planning to use microarrays to study global regulation of gene expression in response to changes in

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the environment of the cells, such as replacement of glucose by a non-preferred source of carbon or oxygen by a non-preferred electron acceptor, should follow the example set by this investigation and attempt to achieve the condition of gratuity required for an unambiguous interpretation of their results.

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